

Attorney Docket No.: **PENN-0857**  
Inventors: **Eberwine and Madison**  
Serial No.: **10/630,333**  
Filing Date: **July 30, 2003**  
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This listing of the claims will replace all prior versions and listings of claims in the application:

**Listing of the claims:**

Claim 1 (currently amended): An improved method of directional cloning of DNA comprising:

- (a) generating single stranded (-) cDNAs;
- (b) annealing a first oligonucleotide encoding a restriction site to the single-stranded (-) cDNAs, wherein the first oligonucleotide encodes a restriction site flanked on both its 5' and 3' ends with at least two degenerate nucleotides, to create double-stranded regions on the single-stranded (-) cDNAs;
- (c) cleaving the single-stranded (-) cDNAs with double-stranded regions of step (b) with a cognate restriction enzyme so that regions to be replicated in a second (+) strand synthesis are limited; and
- (d) directly ligating the cleaved regions of double stranded cDNAs of step (c) into a cloning or expression vector to clone the DNA.

Claim 2 (currently amended): The method of claim 1 further comprising annealing a second oligonucleotide encoding the restriction site and having a homopolymeric tail complementary to a homopolymeric tail at the 3' end of the single-stranded (-) cDNAs to the 3' end of the single-stranded (-) cDNA strand cDNAs of step (a) to form a stable replication competent gapped single-stranded circle by

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hybridization of the restriction site of the second oligonucleotide to the restriction site of the single-stranded (-) cDNAs.